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Crosslinked glucose oxidase and horseradish peroxidase can mimic the macrophage oxidative function in vivo in respect to tumor cells as targets. We used crosslinked glucose oxidase, horseradish peroxidase, and luminol-bovine serum albumin entrapped in filter paper disks to study the chemiluminescent properties of this macrophage-mimicking material. The disks showed a sensitivity to D-glucose down to 1 ug/ml. The luminescence was driven specifically by glucose at pH 6.0, 6.5, 7.0, 7.4, 8.0, and 10.0. The best signal-to-noise ratio, 44,705 to 1, was achieved at pH 7.4. Superoxide dismutase enhanced the chemiluminescence and catalase suppressed it. Therefore, superoxide was produced by the crosslinked system, and the chemiluminescence was dependent on hydrogen peroxide.

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GEL STATE CHEMILUMINESCENCE: AN ARTIFICIAL ELECTRON TRANSPORT SYSTEM*

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INTRODUCTION

Crosslinked glucose oxidase (GO) and horseradish peroxidase (HRP) can mimic macrophage oxidative function in vivo in respect to tumor cells as targets.[1-3] Both the flavoprotein of NADPH oxidase of macrophages and GO contain flavin adenine dinucleotide as their prosthetic group.[4] HRP has a noncovalently bound hematoporphyrin IX prosthetic group as does the cytochrome b_{559} (green hemoprotein) of NADPH oxidase.[4] Therefore, we anticipated a similarity in biochemical mechanism of action and chemiluminescence (CL) between the semi-synthetic crosslinked GO and HRP and the macrophage NADPH oxidase.

RESULTS AND DISCUSSION

We crosslinked 25 µl of GO (1 mg/ml), HRP (1 mg/ml), and luminol (LH_2)-bovine serum albumin (30 mg/ml) with 0.5% glutaraldehyde in pH 6.9 PBS in a 7-mm diameter filter paper disk to study the CL properties of this macrophage mimicking material (IC-1L).[1,2,5]

Table 1 shows that the CL (recorded with a Turner Designs 20e luminometer) of the disks was driven specifically by glucose (200 µl of 0.5 mg/ml) at pH 6.0, 6.5, 7.0, 7.4, 8.0, and 10.0. The best signal-to-noise ratio, 44,705 to 1, was achieved at pH 7.4. Even without luminol, there was considerable CL from IC-1 (189 to 1 signal-to-noise ratio) when disks treated with glucose were compared with those without glucose at pH 7.4.

The kinetics of the CL also varied with age and handling of the disks. Disks produced maximum CL on the second day after preparation, as did those in Table 1. The peak CL was 6 minutes after activation and persisted for about 3 minutes. On the first day, third day, and subsequent days after synthesis and storage at 4°C, the peak CL of the disks was replaced by a plateau at a lower maximum CL that was achieved slowly

Table 1. Chemiluminescence (relative light units/45 secs ± S.D. after 6 min) of Crosslinked Glucose Oxidase, Horseradish Peroxidase, and Bovine Serum Albumin (IC-1) or Luminol-Bovine Serum Albumin (IC-1L)

pH[a]	IC-1	IC-1L
6.0	0.365 ± 0.0146(n=5)	6.851 ± 0.862(n=6)
6.5	0.370 ± 0.0204(n=5)	16.76 ± 1.778(n=7)
7.0	1.283 ± 0.102(n=5)	112.4 ± 1.329(n=6)
7.4	1.477 ± 0.181(n=4)	348.7 ± 11.57(n=7)
7.4(NG)[b]	---	0.0078 ± 0.0055(n=5)
8.0	1.773 ± 0.337(n=4)	1998.0 ± 228.5(n=6)
8.0(NG)	---	0.4865 ± 0.008(n=6)
10.0	1.045 ± 0.068(n=4)	2397.0 ± 197.0(n=7)
10.0(NG)	---	0.8265 ± 0.011(n=6)

[a]The buffer was phosphate buffered saline (0.01 M sodium phosphate and 0.154 M NaCl) with 0.5 mg/ml D-glucose; at pH 8.0 and 10.0 the PBS pH was adjusted with 0.01 M sodium carbonate.
[b]NG = PBS without glucose.

and persisted for 6 to 10 minutes (Figure 1). Also, the spontaneous CL of IC-1L increased with time as the disk remained at room temperature in pH 7.4 PBS. Luminometry with the Quantitative Luminescence Imaging System (QLIS, USAF) indicated that intense localized long-term (30 minutes or more) CL was produced by IC-1L when compared with standard (1 x 10^{-7} M to 1 x 10^{-6} M) luminol in dimethyl sulfoxide solutions activated by 0.1 N NaOH (1-2 second CL lifetimes).

In Table 2, we report that the increase in bulk buffer pH around the IC-1L gel (a change of 2.6 units) led to an increase of only 1.68 pH

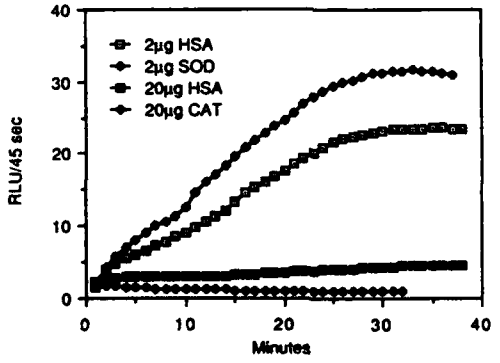

Figure 1. Chemiluminescence of IC-1L disks at room temperature. Each disk was either activated with 200 µl of pH 7.4 PBS containing 100 µg D-glucose or treated with PBS without glucose.

Table 2. Buffering Capacity and Proton Exchange by IC-1L Disks

Components[a]	pH ± S.D.	
Buffer (no gel)	7.4	10.0
Bulk solution		
with gel	7.61 ± 0.04(n=4)	9.09 ± 0.12(n=3)
with gel and glucose	7.66 ± 0.05(n=4)	9.12 ± 0.12(n=3)
Gel surface		
no glucose	6.11 ± 0.07(n=8)	6.98 ± 0.02(n=3)
glucose	5.42 ± 0.09(n=4)	6.90 ± 0.03(n=3)

[a]Each 7-mm paper disk containing 25 µl of gel was treated with 200 µl buffer (0.01 M PBS at pH 7.4 or 0.01 M TRIS/saline at pH 10.0) with or without 0.5 mg/ml D-glucose.

units in the surface of activated gel and 1.76 units in gel without glucose. The gel acted like a buffer with a pKa near 5 or 6 as indicated in Table 2. The addition of glucose consistently decreased the surface pH in either pH 7.4 or pH 10.0 buffer. These data indicate that the polyanionic gel entraps protons (hydrogen ions) even though the bulk solution may be very basic. Activation of CL facilitates the accumulation of protons in the gel.

Figure 2 illustrates the nonspecific and specific antioxidative response of IC-1L to inert human serum albumin (HSA), superoxide dismutase (SOD), and catalase (CAT). Comparing Figures 1 and 2, one can see that at 2 µg, HSA does not affect CL. However, at 2 µg, SOD enhances the CL. At 20 µg, HSA quenches CL by 81%, but CAT suppresses CL by 96%. These data indicate a strong participation of H_2O_2 and a minor but significant abortive role of free $\cdot O_2^-$. Dismutation of released $\cdot O_2^-$ facilitates the CL.

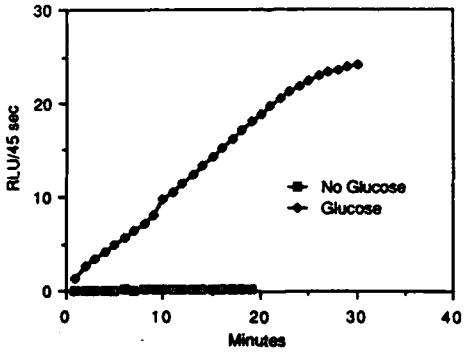

Figure 2. Chemiluminescence of IC-1L disks activated as described in Figure 1, but with human serum albumin, superoxide dismutase, or catalase.

CONCLUSIONS

Based on the data we present here, we conclude that the CL mechanism of IC-1L is as follows (where LO_2 is peroxyluminol or cyclooxyluminol and AP^{-2} is aminophthalate):

$$2\ \text{D-glucose} + 2O_2 \rightarrow 2\ \text{D-gluconic acid} + 2H_2O_2 \quad (1)$$

$$HRP + H_2O_2 + LH_2 \rightarrow HRP(\cdot OH) + H_2O + LH\cdot \quad (2)$$

$$HRP(\cdot OH) + H_2O_2 \Leftrightarrow HRP(\cdot O_2^-) + H_2O + H^+ \quad (3)$$

$$HRP(\cdot O_2^-) + LH\cdot \rightarrow HRP + LO_2 + H^+ \quad (4)$$

$$LO_2 \rightarrow AP^{-2} + N_2 + \text{light} \quad (5)$$

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